Supplemental Data. Grenville-Briggs et al. (2008) Cellulose synthesis in *Phytophthora infestans* is required for normal appressorium formation and successful infection of potato.

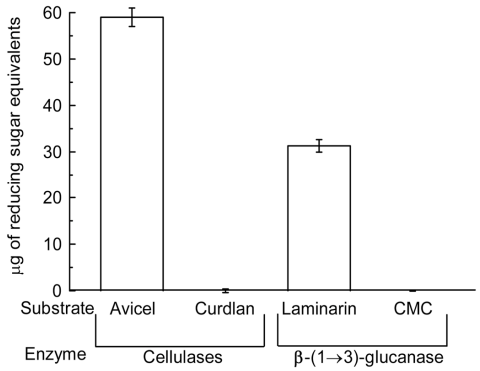


Fig. S1. Specificity of the hydrolytic enzymes used for the quantitative analysis of the cellulose and (1→3)-β-glucan in the walls of wild type and silenced appressoria. The reactions were performed as described in Methods by replacing the extracted cell wall material by 1 mg/mL cellulose (Avicel, carboxymethylcellulose (CMC)) or (1→3)-β-glucan (laminarin, curdlan) substrates. The activities are expressed as µg of reducing sugar equivalents released in the reaction mixture by the hydrolytic enzymes after 24 h reaction.

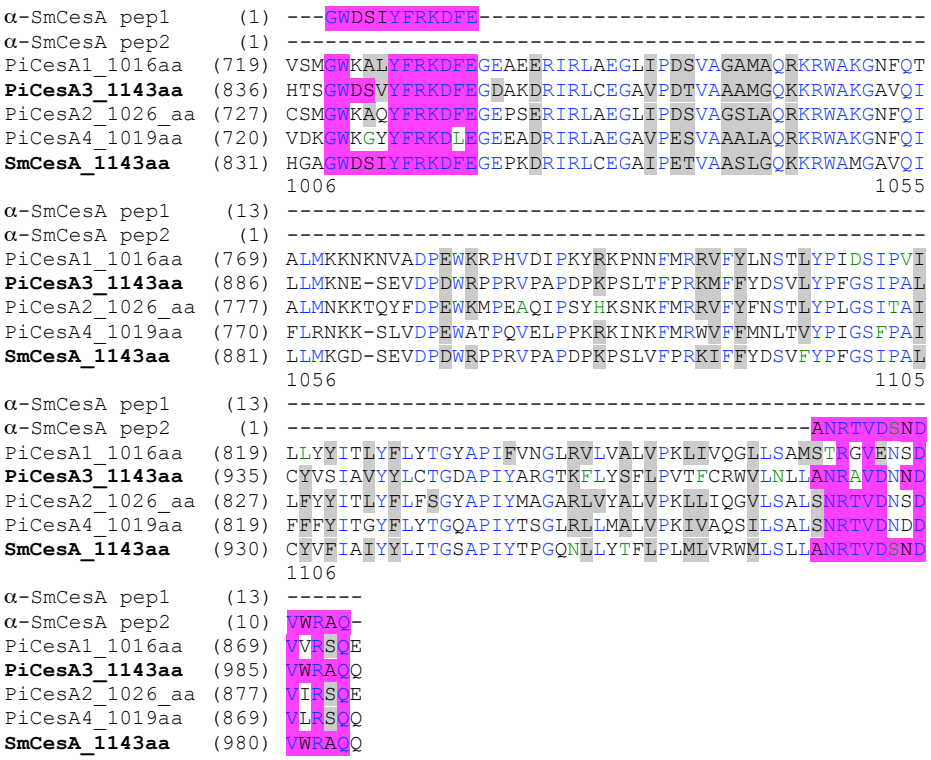


Fig. S2. Alignment of the 4 CesA sequences from *P. infestans* (PiCesA 1-4) with a segment of the CesA protein from *S. monoica* (SmCesA). The sequences and positions of the 2 peptides used to produce anti-CesA antibodies are indicated in the alignment (α-SmCesA pep1 and α-SmCesA pep2). Amongst the PiCesA sequences, PiCesA3 is the closest homologue to SmCesA. This is highlighted in the Figure by using bold font for the names of these 2 proteins.

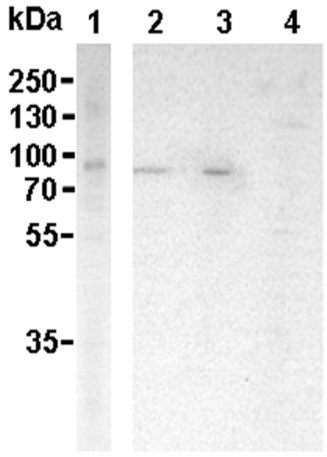
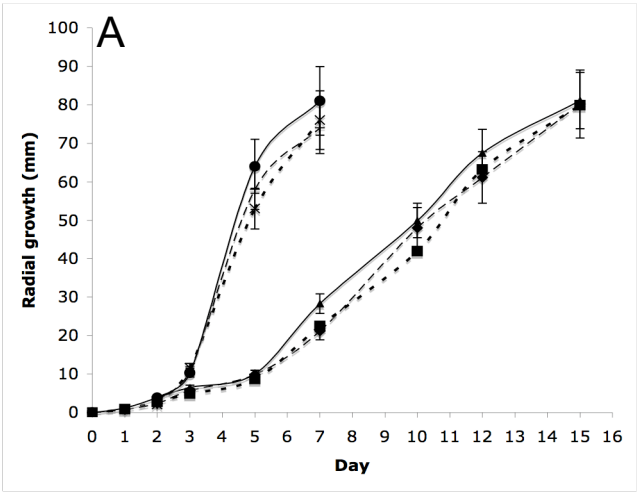
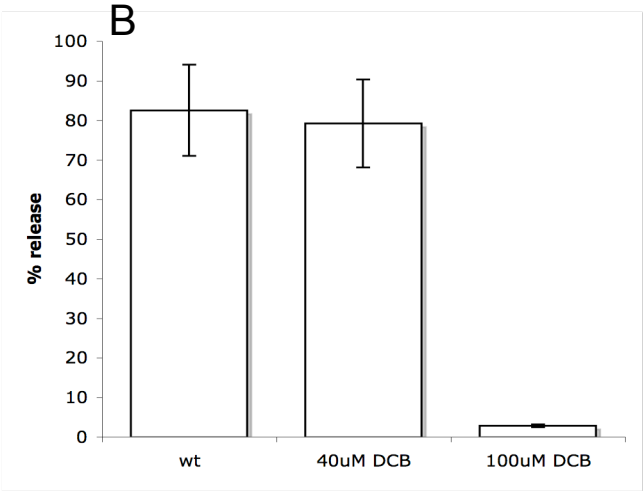
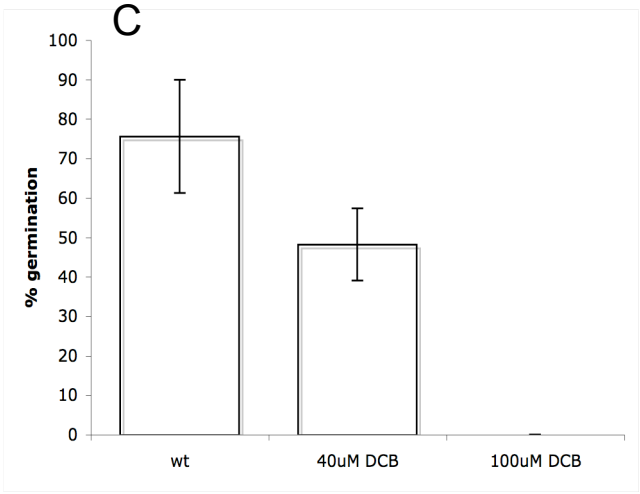
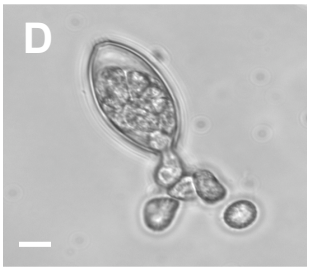


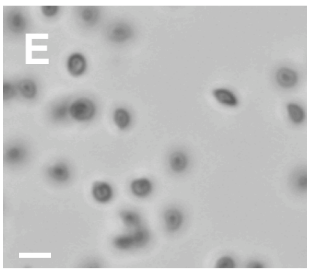
Fig. S3. Western blot analysis of the anti-CesA antibodies. A mixture of the 2 peptides presented in Fig. S2 was used to produce anti-CesA antibodies as described in Methods. The antibodies were used to probe proteins from purified plasma membranes from *S. monoica* (lane 1) and *P. infestans* (lane 2) (see Supplementary Methods for membrane preparation). Total membranes (lane 3) and the cytosolic fraction (lane 4) of *P. infestans* mycelium were probed by the antibodies in the same conditions. Specific signals at the expected molecular weight were obtained with all membrane fractions, but not with the cytosolic fraction. These results demonstrate that the antibodies specifically recognize CesA proteins that are located in purified plasma membranes from both *S. monoica* and *P. infestans*.

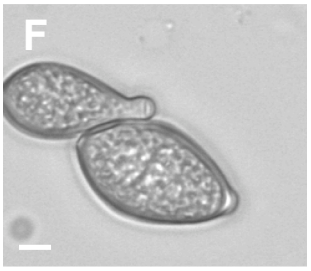

A
Radial growth (mm)
Day
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
0 10 20 30 40 50 60 70 80 90 100

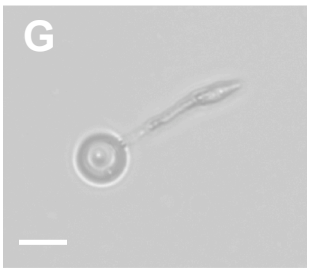

B
% release
wt
40uM DCB
100uM DCB
0 10 20 30 40 50 60 70 80 90 100


C
% germination
wt
40uM DCB
100uM DCB
0 10 20 30 40 50 60 70 80 90 100


D


E


F


G

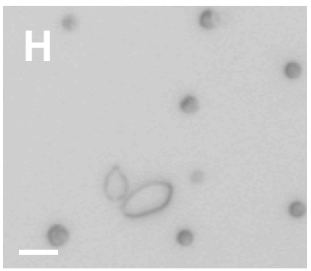

H

Fig. S4. Effects of DCB on radial growth, zoospore release and cyst germination in *P. infestans*.

(A) Radial growth in mm of *P. infestans* grown in media with no treatment (solid lines) in liquid cultures (circular markers) or solid plates (triangular markers) or with the addition of 100μM DCB to liquid medium (broken lines, Cross markers) or 100μM DCB to solid medium (broken lines, diamond markers) or 40μM DCB to liquid cultures (broken lines, star markers) or 40μM DCB to solid cultures (broken lines, square markers). Mean of three replicates, error bars represent confidence intervals calculated for three biological replicates.

(B) Percentage zoospore release from sporangia incubated at 4^{o}C for four hours in water (controls) or DCB at either 40μM or 100μM. Error bars represent confidence intervals calculated for three biological replicates.

(C) Percentage germination of cysts incubated at room temperature for four hours in water (controls) or DCB at either 40μM or 100μM. Error bars represent confidence intervals calculated for three biological replicates.

(D) Light micrograph showing wild-type sporangia releasing zoospores in water.

(E) Light micrograph showing wild-type zoospores released and swimming after four hours incubation in water at 4^{o}C.

(F) Light micrograph showing uncleaved sporangia after four hours incubation in 100μM DCB at 4^{o}C.

(G) Light micrograph showing germinated cyst after encystment and four hours of incubation in water at room temperature.

(H) Light micrograph showing ungerminated cysts after encystment and four hours of incubation in 100μM DCB at room temperature.

Scale bars represent 10μM in D-G and 20μM in H.

Supplemental Methods

Isolation of plasma membranes.

Pea broth and Machlis medium (Machlis, 1953) were used to grow mycelium from *P. infestans* and *S. monoica*, respectively. The *S. monoica* and *P. infestans* mycelia were collected after 3 and 5 days of culture (respectively) from 3 Petri dishes containing 100 mL culture medium and ~20 agar plugs as inoculum. The cells were washed with water and dried under vacuum on filter paper. All following steps were performed at 4°C. The cells were homogenized in extraction buffer (Tris-HCl 10 mM pH 7.4) using a Waring blender (4 periods of 10 s at maximum speed). Cytosolic and total membrane fractions were obtained by differential centrifugation (Bulone et al., 1990). Total membranes were resuspended in 5 mM potassium phosphate buffer pH 7.5 containing 0.33 M sucrose, 0.1 mM EDTA and 0.5 mM dithiothreitol (DTT), in order to reach a final protein concentration of 5 $mg.mL^{-1}$ (Bradford (1976) assay, Bio-Rad reagent). Plasma membranes were then purified by two-phase partitioning essentially as described earlier (Larsson et al., 1994) except for the following modifications. The two-phase system was composed of 6% (w/w) dextran T500, 6% (w/w) polyethyleneglycol (PEG) 3350, 5 mM KCl, 0.33 M sucrose, 0.1 mM EDTA, 0.5 mM DTT and 5 mM potassium phosphate buffer pH 7.5 (final concentrations). The purified plasma membranes obtained by repeating the phase partitioning 3 times were washed and resuspended in extraction buffer. The purity of the plasma membranes was assessed by measuring the activity of enzymatic markers of endoplasmic reticulum (cytochrome c reductase) (Briskin et al., 1987), mitochondria (cytochrome c oxydase) (Briskin et al., 1987), and plasma membrane ((13)-β-D-glucan synthase) (Girard and Fèvre, 1984).

Western blot analysis.

Western blot analysis was performed using a classical procedure. Briefly, a theoretical amount of 6 μg protein (Bradford (1976) assay) was used for each sample for SDS-PAGE separation. The proteins were transferred to nitrocellulose membranes at 60 V for 7 h, using a wet transfer chamber from Bio-Rad. Membranes were blocked overnight in 5% non-fat dried milk solubilized in TBS-Tween buffer. The anti-CesA antibodies were used at a 1/500 dilution in 0.5% milk in TBS-Tween. After washing, the membranes were incubated in a 1/3000 dilution of anti-rabbit IgG antibodies conjugated to horseradish peroxidase (GE Healthcare, cat # NA934). The detection was performed using the ECL Western blotting detection system from GE Healthcare (cat # RPN2109) following the manufacturer's instructions.

Effects of DCB on radial growth, zoospore release and cyst germination.

DCB at either 40μM or 100μM final concentration was added to liquid or solid rye media and 90mm plates were inoculated with a single 1.5cm circular plug of *P. infestans* cut from a stock plate using a cork borer to ensure equal sized plugs were used throughout the experiment. Radial growth was measured as the longest mycelial growth in millimetres from the edge of the plug and recorded until the mycelium had reached the edge of the plate.

Zoospores were released from sporangia and cysts germinated as described previously in (Grenville-Briggs et al., 2005) with the following differences, sporulating cultures were flooded with chilled water, or DCB at 40μM or 100μM and left to release zoospores at 4^{o}C for four hours. Percentage release was recorded. To induce encystment zoospores were shaken vigorously for 2 minutes. Cysts were collected and resuspended at 1x10^{5}ml in water or DCB at 40μM or 100μM and left to germinate at room temperature for up to four hours. Percentage germination was recorded. All experiments were carried out in triplicate.

Supplemental References

Bulone, V., Girard, V., and Fèvre, M. (1990) Separation and partial purification of (1→3)-β-glucan and (1→4)-β-glucan synthases from *Saprolegnia*. Plant Physiol. **94,** 1748-1755.

Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72,** 248-254.

Briskin, D.P., Leonard, R.T., and Hodges, T.K. (1987) Isolation of the plasma-membrane: membrane markers and general principles. Methods Enzymol. **148,** 542-558.

Girard, V., and Fèvre, M. (1984) β-1,4 and β-1,3 glucan synthases are associated with the plasma membrane of the fungus Saprolegnia. Planta **160,** 400-406.

Larsson, C., Sommarin, M., and Widell, S. (1994) Isolation of highly purified plant plasma membranes and separation of inside-out and right-side-out vesicle. In Walter, H., and Johansson, G. (Eds). *Aqueous Two-Phase Systems*. San Diego: Academic Press, pp. 451-459.

Machlis, L. (1953) Growth and nutrition of watermolds in the subgenus Euallomyces. II – Optimal composition of the minimal medium. Am. J. Bot. **40,** 449-460.